Uptake of cadmium by lettuce in tropical contaminated soils

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Abstract
Cadmium (Cd) is one of several metals that have come under suspicion in recent years as environmental contaminants potentially harmful to human health. The objective of this study was to evaluate the availability of Cd in soil and its distribution in root, stem and leaf of lettuce plants grown in a soil contaminated with Cd. The experiment was conducted under greenhouse conditions in Piracicaba-SP. Lettuce plants (two plants/pot) were cultivated in pots filled with 3/dm^3 of Oxisol samples using a randomized block design in a factorial scheme (5 x 2) with three replicates. The treatments consisted of: 0.0; 0.5; 1.3; 3.0 and 6.0 mg/dm^3 rates of Cd (as $CdCl_2$), based on the guideline established by the Environmental Agency of the State of Sao Paulo, Brazil - Cetesb. The external loading of Cd increased available Cd in soil as estimated by three extractors: DTPA, Mehlich-1 and $CaCl_2$. The concentration of Cd accumulated in different parts of plants was positively related to available Cd in the soil and to Cd application rates. The current “prevention” values of soil contamination for Cd proposed by Cetesb were somewhat restrictive but may still result in Cd concentration in the edible part of lettuce above the permitted value by food legislation.

Key Words
Lactuca sativa L., Cd availability, food safety, soil pollution, trace-element

Introduction
Cadmium is a heavy metal naturally present in soils. It may be added to the soil as a contaminant in fertilizers, manure, sewage sludge and from aerial deposition. The amount of cadmium contributed from each source varies with location due to differences in soil types, management practices and exposure to pollution sources, but the level of Cd in soil appears to be increasing over time (Kabata-Pendias and Mukherjee 2007). Cadmium is of concern to human health because it is easily transferred to human beings through food chain. Cadmium has no essential biological functions and highly toxic to plants and animals. Human dietary intake of Cd from vegetables is primarily dependent on the concentration of Cd in the edible part and not necessarily on the uptake of Cd by the whole plant (Yang *et al.* 2004). To evaluate the soil quality, Brazilian environmental protection agencies used generic Cd values found in bibliographic references; these values are often obtained under different conditions from those found in the tropical regions, and thus may lead to erroneous hazard estimates. The objective of this study was to evaluate the availability of Cd in soil and its distribution in root, stem and leaf of lettuce plants grown in the Cd contaminated soil.

Methods
A greenhouse experiment was carried out from November 2008 to March 2009 in Piracicaba, State of São Paulo, Brazil. Lettuce (*Lactuca sativa* L.) cv. Elisa da Sakata was used as test plant. The experimental design was randomized complete blocks in a factorial scheme 5 x 2 (five rates of Cd and two soils) with three replications, totaling 30 experimental units. Each experimental unit consisted of a pot filled with 3/dm^3 of two Oxisols (Typic Haplustox and Rhodic Hapludox), with chemical and physical attributes presented in Table 1. The treatments consisted of: 0.0; 0.5, 1.3; 3.0 and 6.0 mg Cd/dm^3 soil, based on the guideline established by Cetesb (2005), added as $CdCl_2.H_2O$. The soils with and without Cd loading were incubated at room temperature for 60 days with soil moisture maintained at 60% water holding capacity. Adequate amounts of $CaCO_3$ and $MgCO_3$ (Ca:Mg ratio of 3:1) were added to increase soil base saturation (BS = 80%) (Trani *et al.* 1997). Only the Typic Haplustox was fertilized (basal application and side-dressing) according to Malavolta (1980). Two lettuce plants were grown per pot and harvested on the 40th day after emergence. Root, stem and leaf were separately harvested and washed two to three times with tap water and once with deionized water before being oven dried at 65 °C for 72 hr. The dry matter yields of each plant part were recorded, and oven dried plant tissues were ground in a Wiley mill, and submitted to nitric-perchloric

digestion according to Malavolta *et al*. (1997) for determination of Cd by inductively coupled plasma mass spectroscopy (ICP-MS). Immediately before plant transplanting soil samples from each pot were collected and analyzed for available Cd by using 0.1 mol L^{-1} $CaCl_2$ (Houba *et al.* 2000), DTPA (pH 7.3) (Abreu *et al.* 2001) and Mehlich-1 (Embrapa 1997). Data were submitted to analysis of variance and polynomial regression, using the SAS system of analysis (SAS Institute 2002). Correlation coefficients were determined between the Cd concentration in soil and Cd accumulated in leaf.

Table 1. Chemical[A] and physical[B] attributes of the soils (0-20 cm depth) used in the experiment.

Attributes	Rhodic Hapludox	Typic Haplustox
pH $CaCl_2$ 0.01 M	5.7	4.1
Organic matter (g/dm^3)	51	26
P (mg/dm^3)	103	5
K ($mmol_c$/dm^3)	10.3	0.7
Ca^{+2} ($mmol_c$/dm^3)	62	6
Mg^{+2} ($mmol_c$/dm^3)	18	4
Al^{+3} ($mmol_c$/dm^3)	2	7
H+Al ($mmol_c$/dm^3)	25	42
Sum of bases ($mmol_c$/dm^3)	90.3	10.7
CEC ($mmol_c$/dm^3)	115,3	52.7
Base saturation (%)	78	20
Al saturation (%)	2	40
B (mg/dm^3)	0.65	0.11
Cu^{+2} (mg/dm^3)	2,2	0.5
Fe^{+2} (mg/dm^3)	32	71
Mn^{+2} (mg/dm^3)	47.2	3.7
Zn^{+2} (mg/dm^3)	9.5	0.7
Cd^{+2} (mg/dm^3)	<0.01	0.01
Particle size (%)		
Sand (> 0.05 mm)	41	64
Silt (> 0.002 and < 0.05mm)	14	16
Clay (< 0.002 mm)	45	20

CEC = cation exchange capacity. [A]Raij *et al*. (2001). [B]Embrapa (1997).

Results

Extractable Cd estimated by the three extraction procedures increased significantly with increasing rate of external Cd loading (Figure 1a, b, c). The relationship between extractable Cd and Cd loading rates followed a linear model for both soils. Among the three extractants, DTPA and Mehlich-1 extracted similar amount of labile Cd in soils, and the 0.1 mol L^{-1} $CaCl_2$ extraction provided the least amount of labile Cd with a relatively smaller correlation coefficient for one soil (Figure 1c). Therefore, it may not be as good as the other two extraction methods for estimation of available Cd in the clayey tropical soils. Similar results were also reported by Trombeta *et al.* (2009). The concentrations of Cd in root, stem or leaf were linearly or quadratically correlated with external loading rate of Cd (R^2>0.99, p<0.01) (Figure 2a, b, c). Except for root Cd concentration in the Rhodic Hapludox, the relationship between Cd concentration in root, stem or leaf lettuce and Cd application rate fitted a quadratic model. These findings are in agreement with previous reports by Moustakas *et al.* (2001). Leaf Cd concentrations were 16.3 and 35.5 mg/kg at the Cd loading rate of 1.3 mg/dm^3 (alert value) and 45.5 and 76.0 mg/kg at the Cd loading rate of 3.0 mg/dm^3 (intervention value) respectively, for Rhodic Hapludox and Typic Haplustox soil. In general, fresh lettuce contains up to 97% water, the leaf Cd concentrations on the fresh weight basis varied between 0.48 and 2.28 mg/kg and the high end is above critical level of 1.0 mg/kg fresh weight (Anvisa, 1965) or 0.66-3.0 mg/kg on the dry weight basis (Kabata-Pendias and Pendias 2001).

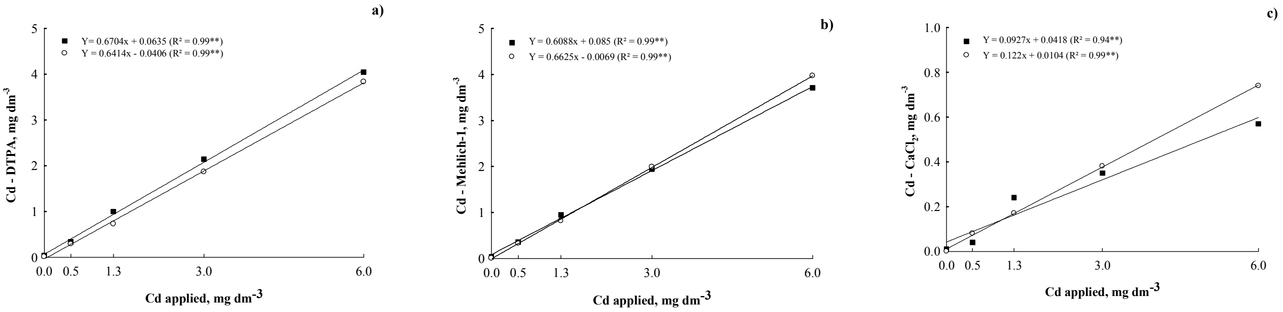

Figure 1. Cadmium concentration in soil extracted by DTPA (a), Mehlich-1 (b) and $CaCl_2$ (c) in function to soil types (■ Rhodic Hapludox; ○ Typic Haplustox), and rates of Cd applied. ** – significant at *p < 0.01*.

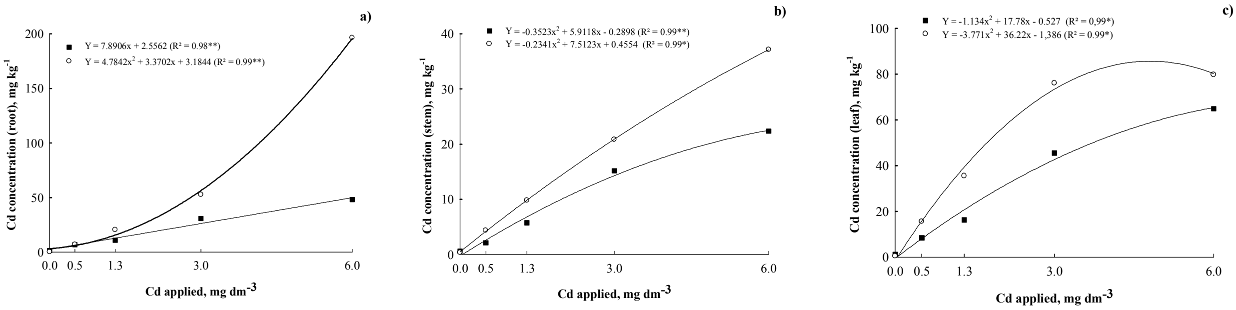

Figure 2. Cadmium concentration in root (a), stem (b) and leaf (c) of lettuce in function to soil types (■ Rhodic Hapludox; ○ Typic Haplustox), and rates of Cd applied. **, * – significant at $p < 0.01$ and $p < 0.05$ respectively.

These results indicate that when available Cd in the soils reached 3.0 mg/dm^3, the concentration of Cd in the lettuce leaf will be high enough to cause risks to human health. Australia has adopted maximum permissible concentrations for Cd in various foods, including 0.05 mg Cd kg^{-1} fresh weight for potatoes (McLaughlin *et al*. 1994). Lettuce is a bioindicator plant of heavy metals because it has the capacity to accumulate high concentrations of metals (Alloway 1995). Lettuce, spinach, celery, and cabbage tended to accumulate high concentrations of Cd, whereas potato tubers, maize, French beans, and peas accumulated relatively small amounts of Cd (Davis and Carlton-Smith 1980). The World Health Organization set a maximum provisional tolerable intake limit of 60 to 70 µg Cd per day for an adult (World Health Organization 1973), and the Codex Alimentarius Commission of FAO/WHO is discussing a limit of 0.1 mg Cd kg^{-1} for cereal grains and oilseeds traded on international markets. However, even small amounts in foods can have a significant effect in the long term because Cd accumulates in the body. There was a positive correlation between leaf Cd accumulation of lettuce and extractable Cd in the soils estimated by each of the three procedures (Figure 3).

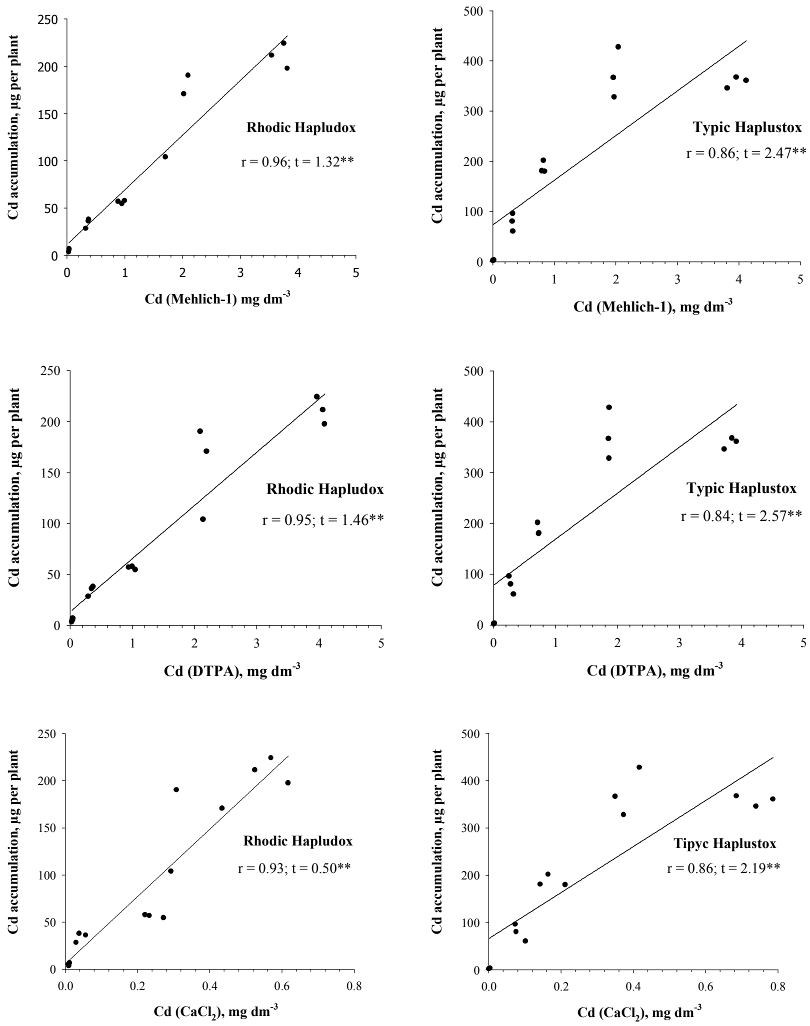

Figure 3. Correlation between leaf Cd accumulation of lettuce and extractable Cd in the soils estimated by Mehlich-1, DTPA and $CaCl_2$. ** – significant at $p < 0.01$.

Conclusion

The external loading of Cd linearly increased available Cd in soils and subsequently Cd concentrations in the root, stem and leaf of lettuce grown in both Oxisols. DTPA, Mehlich-1 and $CaCl_2$ were effective for the determination of available Cd in the tropical soils. The concentrations of Cd in root, stem, and leaf of lettuce plants were significantly increased with an increase in soil available Cd or external Cd loading rate. The current "prevention" values of soil contamination for Cd, proposed by Cetesb, were somewhat restrictive but may still result in Cd concentration in the edible parts of lettuce above the permitted level established by food legislation.

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